Supplementary Information File

RIFAMPICIN NANOFORMULATION ENHANCES TREATMENT OF TUBERCULOSIS IN ZEBRAFISH

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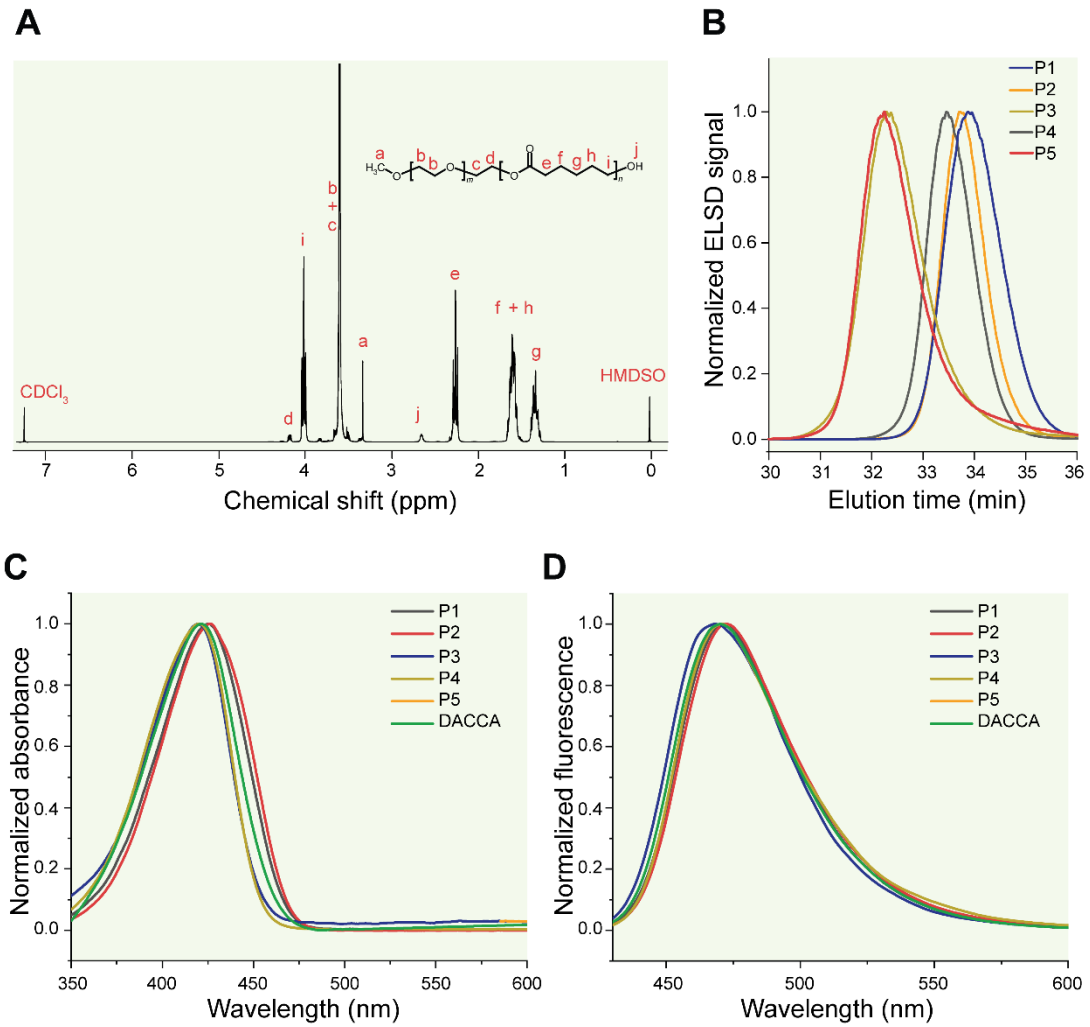

Figure S1. Physico-chemical characterization of prepared MPEO-*b*-PCL copolymers. (**A**) Typical ^{1}H NMR spectrum. Sample MPEO$_{44}$-*b*-PCL$_{18}$ (**P1**) is shown. (**B**) GPC curves of prepared copolymers (**P1**, MPEO$_{44}$-*b*-PCL$_{18}$; **P2**, MPEO$_{44}$-*b*-PCL$_{27}$; **P3**, MPEO$_{44}$-*b*-PCL$_{81}$; **P4**, MPEO$_{113}$-*b*-PCL$_{33}$; **P5**, MPEO$_{113}$-*b*-PCL$_{113}$). (**C**) Normalized absorption spectra of DACCA-labelled copolymers (**P1**–**P5**) and free DACCA in DMSO. (**D**) Normalized fluorescence emission spectra of DACCA-labelled copolymers (**P1**–**P5**) in DMSO (excited at 420 nm).

Table SI. Chemical and molecular weight characterization of MPEO-*b*-PCL copolymers and DACCA content determination.

Copolymer (No.)	**^{1}H NMR** M_n	**GPC**[b] M_w	M_n	$Đ$[c]	**CAC**[d] (μg/mL)	**DACCA**[e] (μg/mg)
$MPEO_{44}$-*b*-PCL_{18}[a] (**P1**)	4 000	6 400	5 500	1.17	8.7	51
$MPEO_{44}$-*b*-PCL_{27} (**P2**)	5 000	6 700	5 400	1.23	5.1	46
$MPEO_{44}$-*b*-PCL_{81} (**P3**)	11 200	18 300	15 200	1.21	9.1	20
$MPEO_{113}$-*b*-PCL_{33} (**P4**)	8 800	8 300	7 200	1.16	17.7	26
$MPEO_{113}$-*b*-PCL_{113} (**P5**)	17 900	19 200	16 000	1.19	8.7	12

[a] Subscript means the repeating units of the block polymer calculated by ^{1}H NMR.
[b] Values were determined by GPC calibrated with polystyrene standards.
[c] Dispersity, M_w/M_n
[d] Critical aggregation concentrations were determined at room temperature in water.
[e] When DACCA-labelled copolymers were used, the content of DACCA was determined by way of UV/VIS spectroscopy.

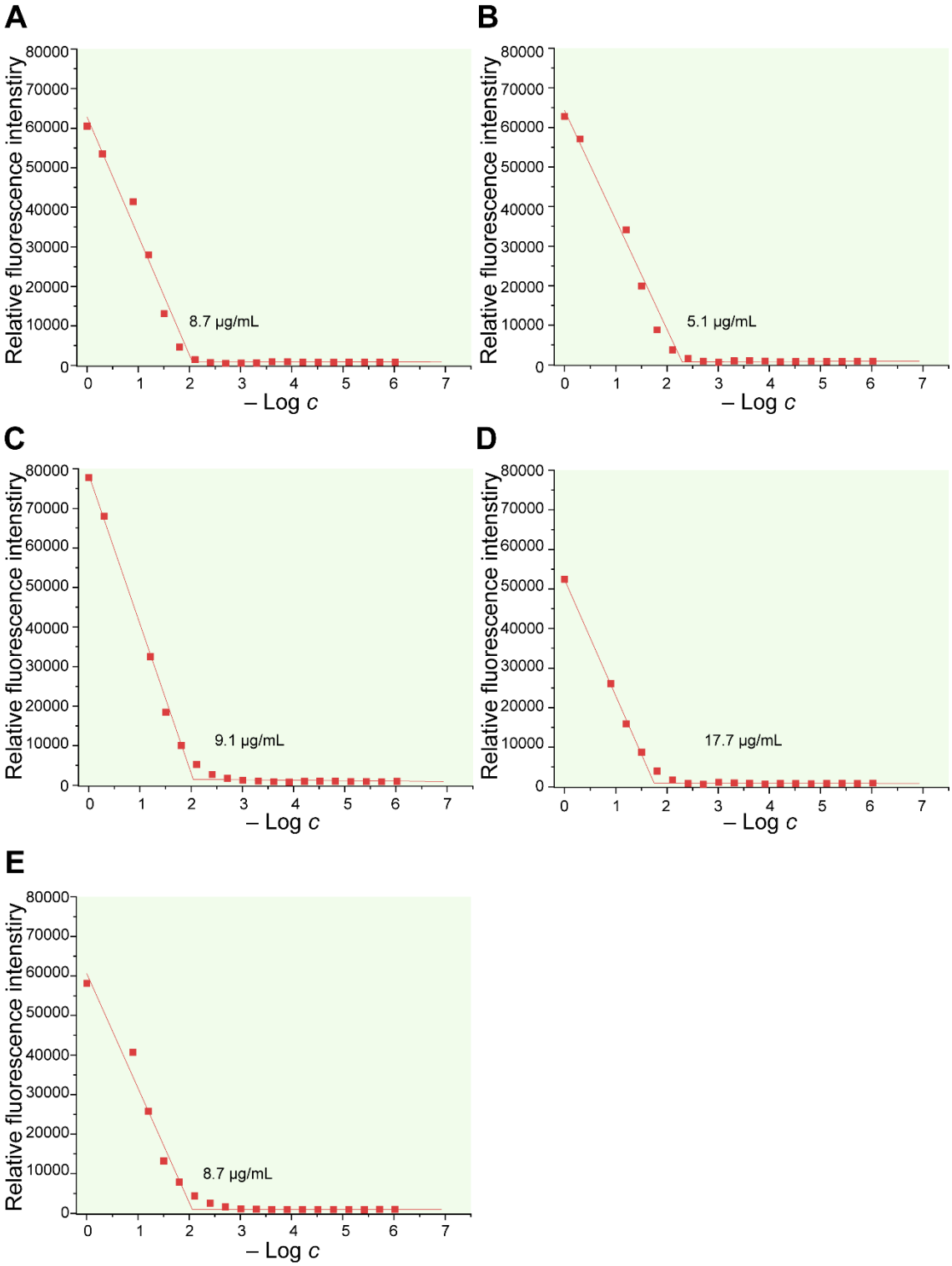

Figure S2. CAC curves found for (**A**) **P1**, (**B**) **P2**, (**C**) **P3**, (**D**) **P4**, and (**E**) **P5** copolymers using Nile red as a fluorescent probe. Relative fluorescence intensities at 650 nm as a function of the concentration of MPEO-*b*-PCL NPs are shown.

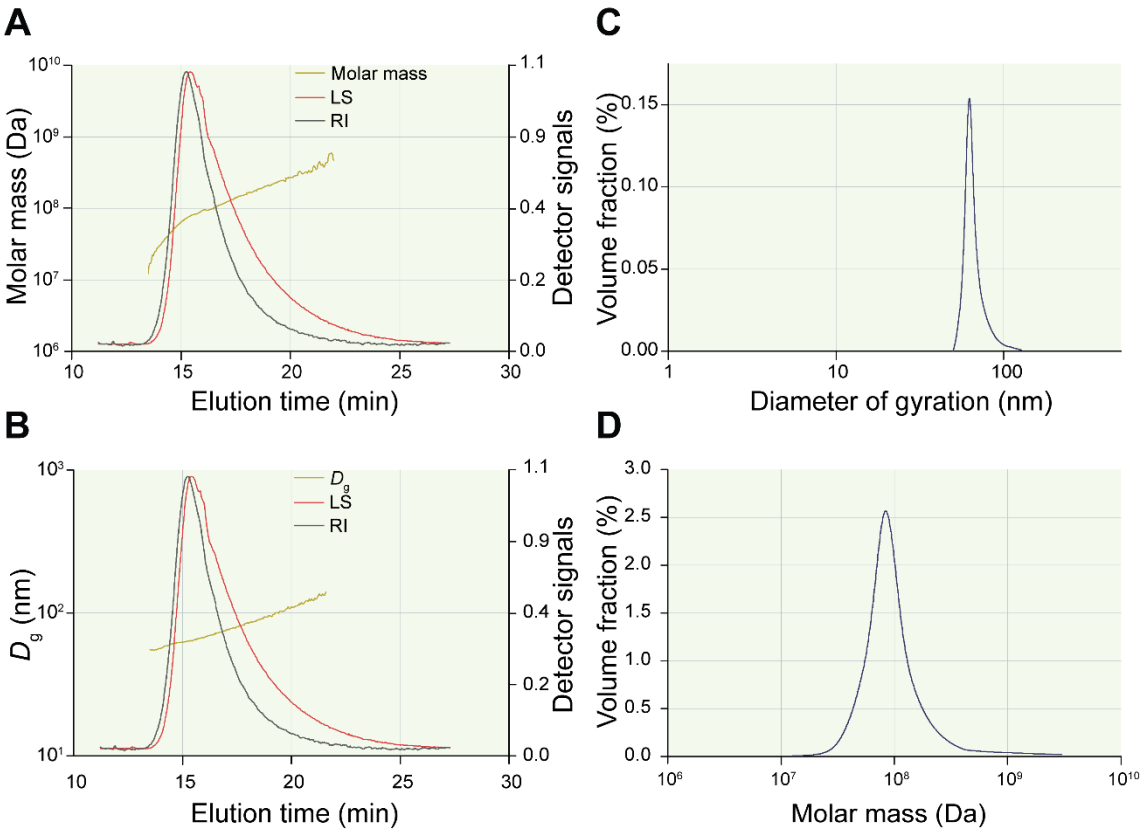

Figure S3. Results from AFFFF analysis of chosen MPEO-*b*-PCL formulation. $MPEO_{44}$-*b*-PCL_{81}-related elugrams with associated molar mass (**A**) and diameter of gyration (**B**); corresponding size (**C**) and molar mass (**D**) volume-weighted distribution functions.

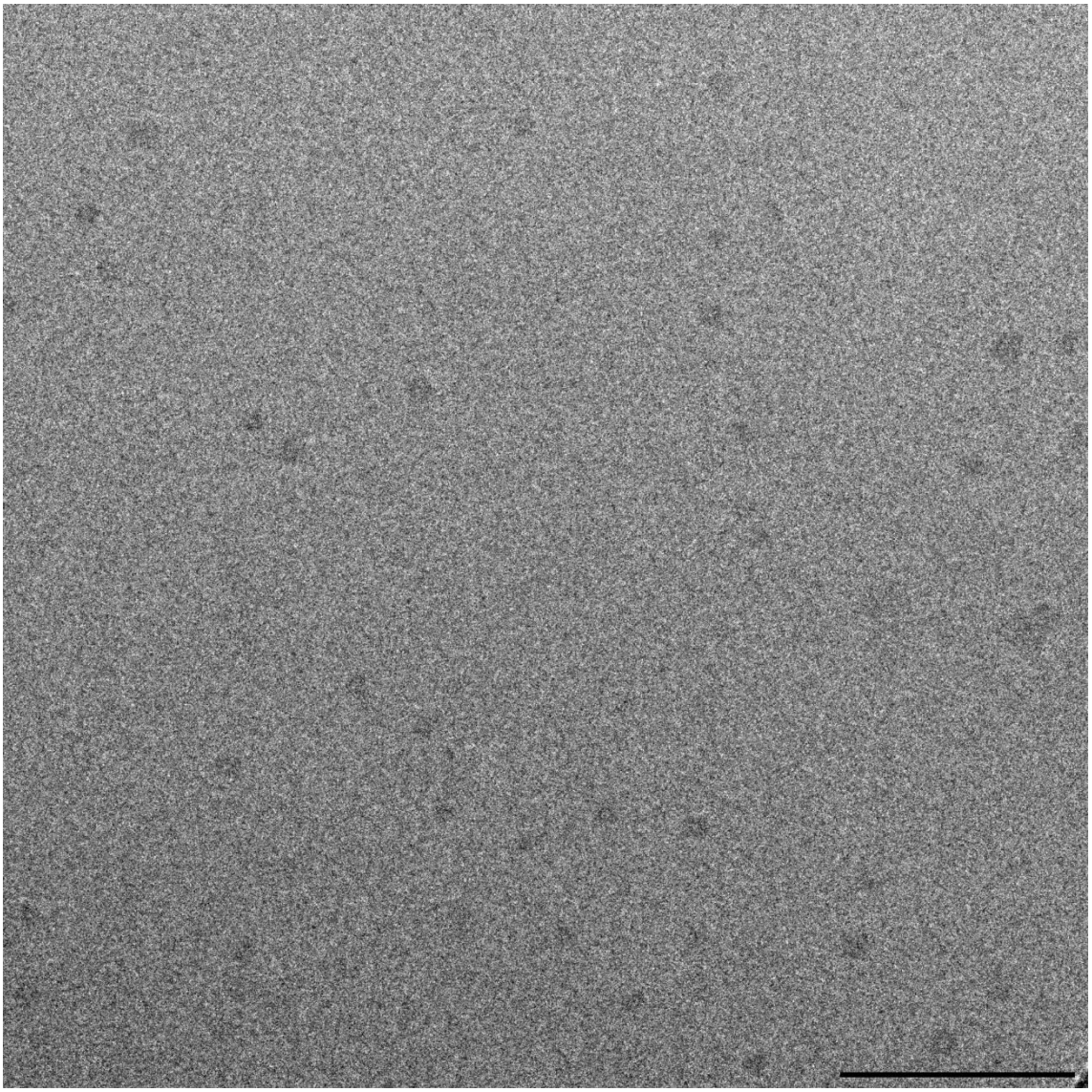

Figure S4. Cryo-TEM image of blank NPs based on $MPEO_{44}$-*b*-PCL_{18} (**P1**). Scale bar: 100 nm.

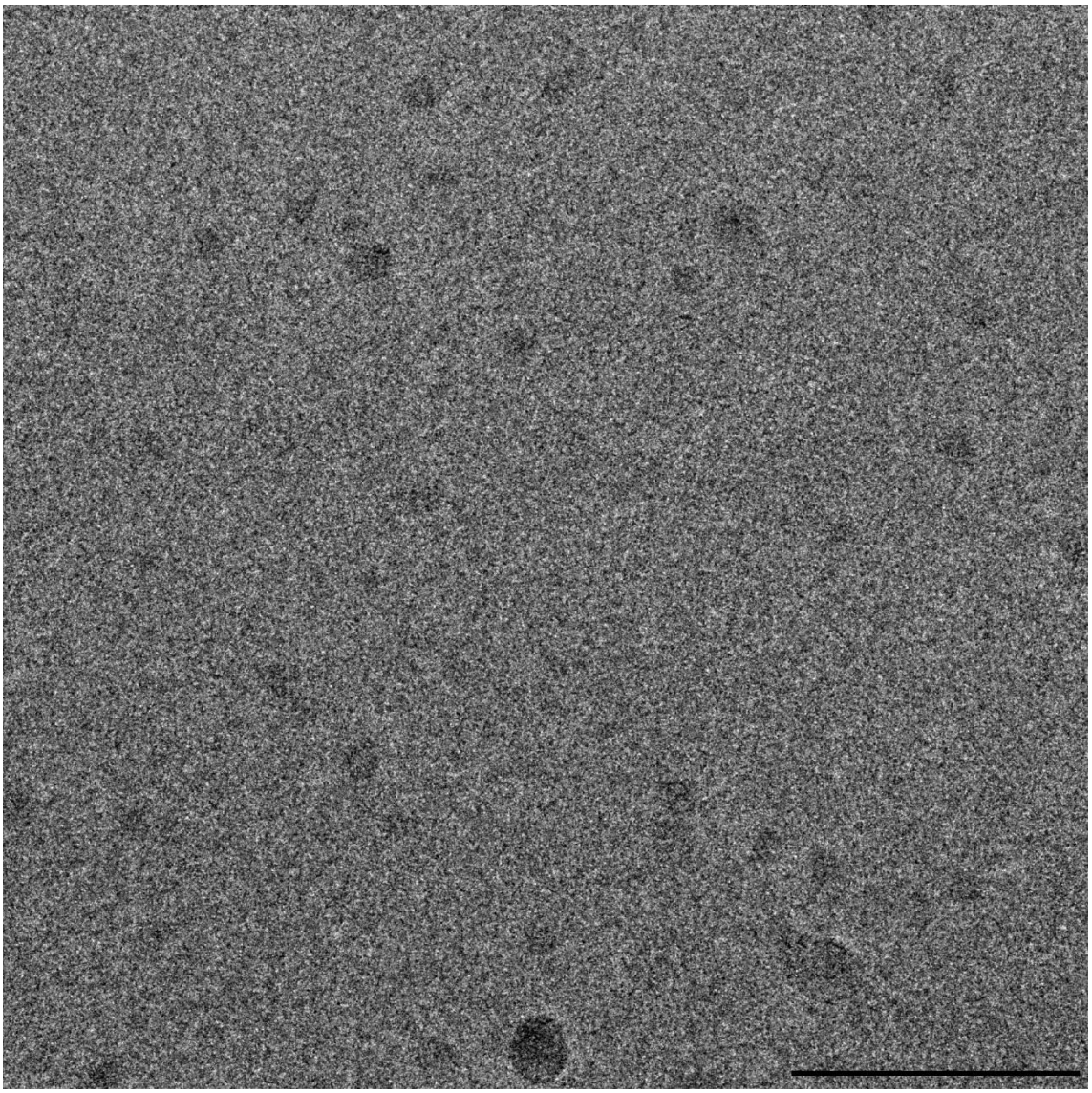

Figure S5. Cryo-TEM image of RIF-loaded NPs based on $MPEO_{44}$-*b*-PCL_{18} (**P1-RIF**). Scale bar: 100 nm.

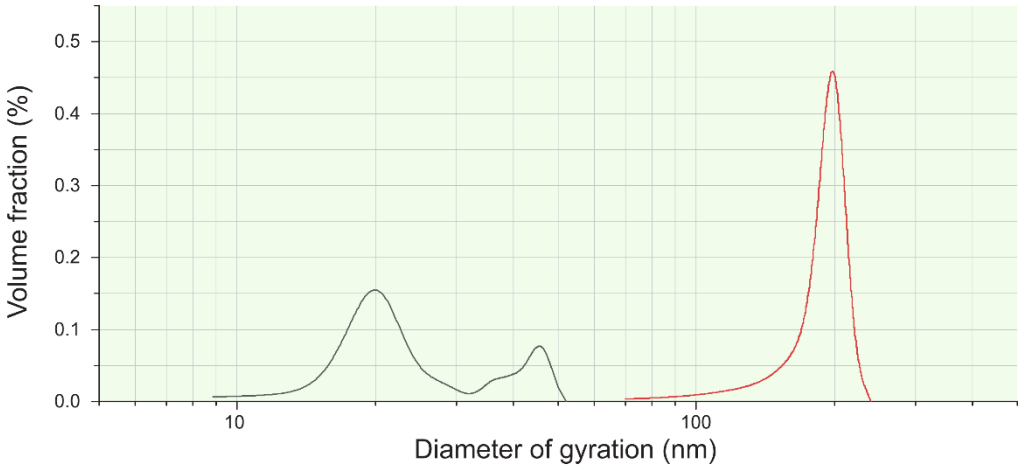

Figure S6. Comparison of volume weighted size distributions of nanoparticles measured with AFFFF method on the freshly prepared **P2** sample ($MPEO_{44}$-*b*-PCL_{27}, gray curve) and on the same sample after 2 months of aging (red curve).

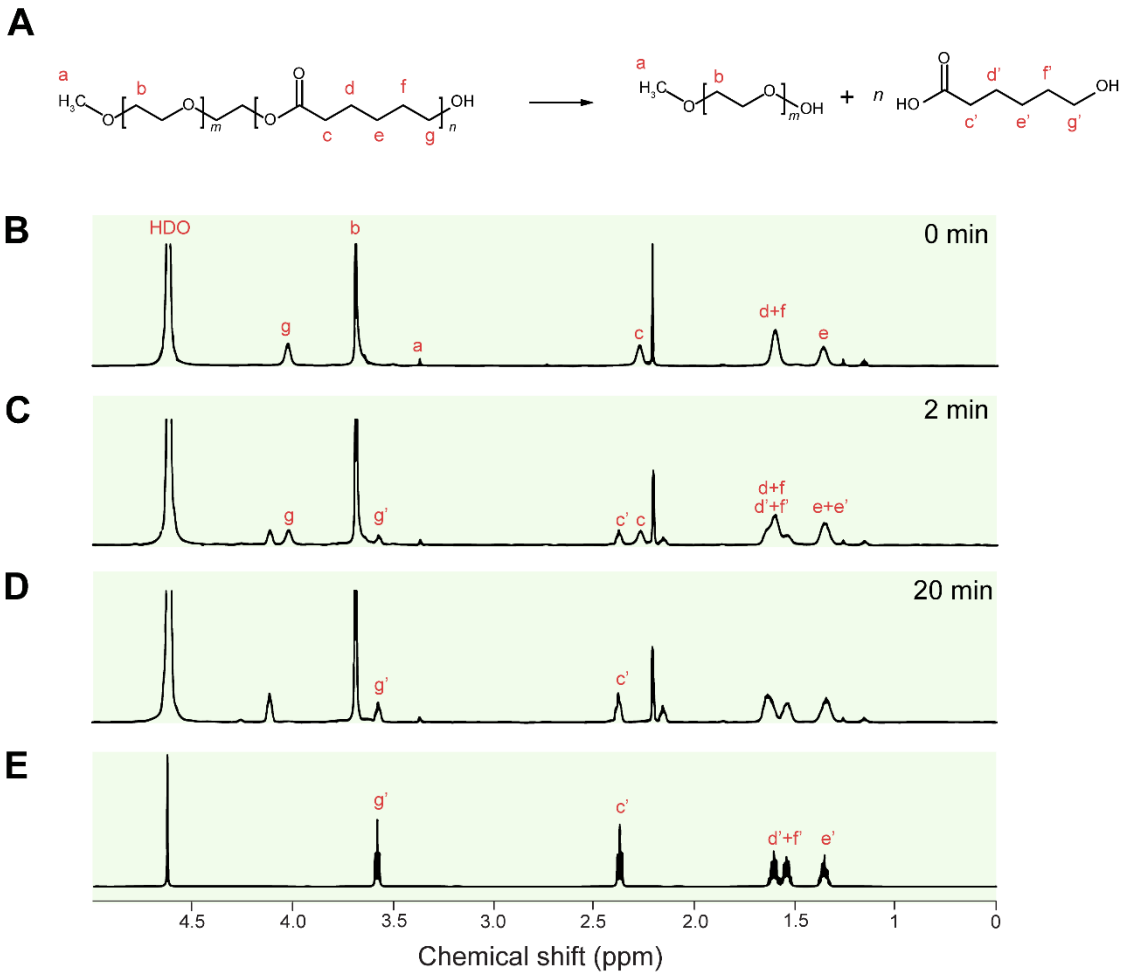

Figure S7. ^{1}H NMR spectra measured in deuterated PBS at 37°C. (**A**) Reaction of lipase-catalyzed degradation of MPEO-*b*-PCL matrix. (**B**) $MPEO_{44}$-*b*-PCL_{27} NPs (**P2**) before lipase addition. (**C**) $MPEO_{44}$-*b*-PCL_{27} NPs (**P2**) after lipase addition (2 minutes). (**D**) $MPEO_{44}$-*b*-PCL_{27} NPs (**P2**) after lipase addition (20 minutes). (**E**) Neat 6-hydroxyhexanoic acid.

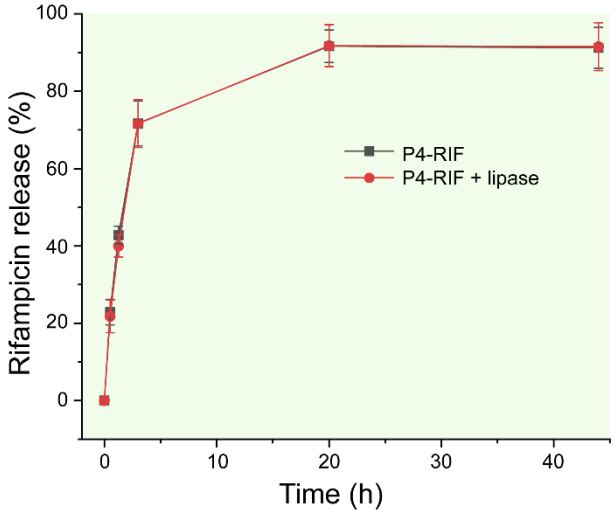

Figure S8. RIF release studied by a common dialysis-based method without and in the presence of bacterial lipase.

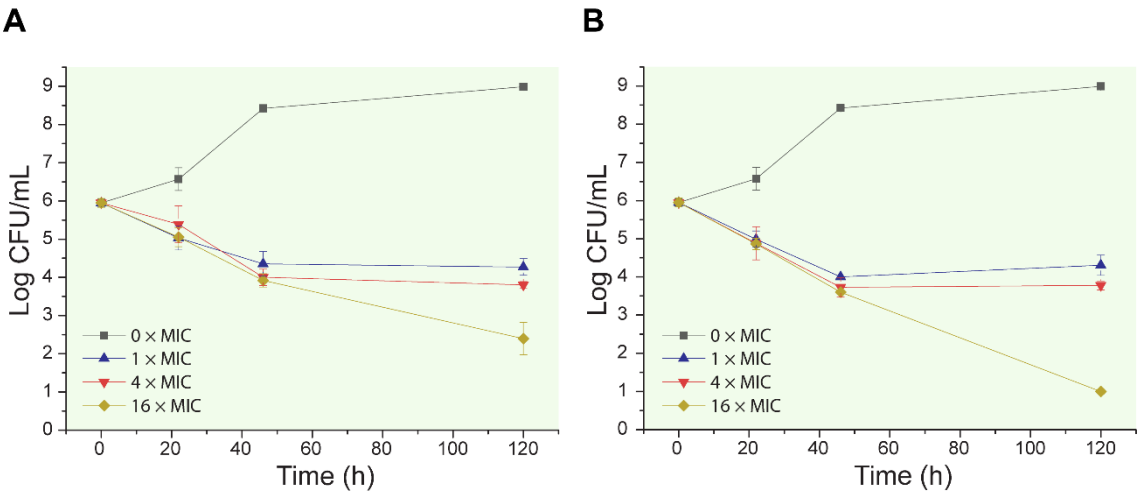

Figure S9. Time-kill curves of *M. fortuitum* exposed to both free RIF (**A**) and **P4**-based NPs loaded with RIF (**B**) in concentrations ranging from 1 × MIC to 16 × MIC estimated for the free RIF (i.e., 8 µg/mL).

Table SII. Cytotoxicity of RIF-loaded NPs in Raw 264.7 macrophages and ZF4 fibroblasts.

Formulation (Copolymer used)	**Cytotoxicity estimated as IC_{50} (μg/mL)**		
	Raw 264.7 (24 h) [a]	**ZF4 (24 h)** [b]	**ZF4 (48 h)** [c]
P1-RIF ($MPEO_{44}$-*b*-PCL_{18})	130.8 ± 4.1	–[d]	–
P2-RIF ($MPEO_{44}$-*b*-PCL_{27})	110.1 ± 11.9	–	–
P3-RIF ($MPEO_{44}$-*b*-PCL_{81})	114.4 ± 12.6	–	–
P4-RIF ($MPEO_{113}$-*b*-PCL_{33})	91.0 ± 2.5	245.6 ± 17.1	290.7 ± 24.3
P5-RIF ($MPEO_{113}$-*b*-PCL_{113})	93.8 ± 6.6	–	–
RIF (Free drug)	295.9 ± 0.7	881.4 ± 199.2	391.3 ± 49.1

[a] Raw 264.7 cells cytotoxicity detected by MTT assay after 24 h.
[a] ZF4 cells cytotoxicity detected by MTT assay after 24 h.
[c] ZF4 cells cytotoxicity detected by MTT assay after 48 h.
[d] Not estimated.

Table SIII. Results from the bio-relevant characterization.

Formulation (Copolymer used)	**Internalization half-live (min)**	**Intracellular degradation half-live (min)**
P1 ($MPEO_{44}$-*b*-PCL_{18})	21.0	51.6
P2 ($MPEO_{44}$-*b*-PCL_{27})	6.2	1,200
P3 ($MPEO_{44}$-*b*-PCL_{81})	11.4	90.0
P4 ($MPEO_{113}$-*b*-PCL_{33})	2.4	1,200
P5 ($MPEO_{113}$-*b*-PCL_{113})	9.4	91.2

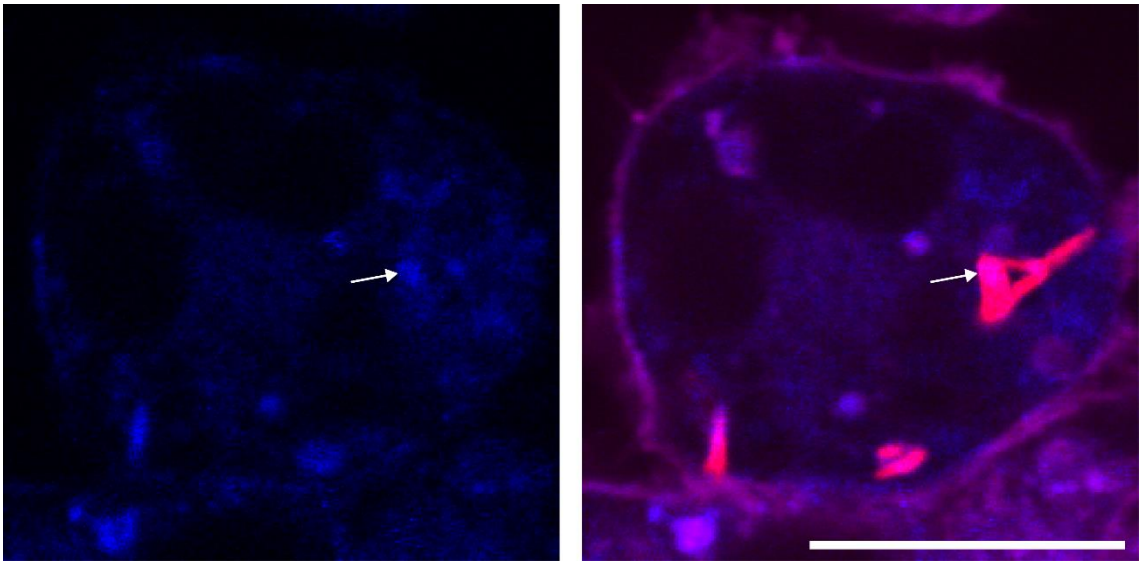

Figure S10. Image from confocal laser scanning microscopy of Raw 264.7 cells after infection by DsRed-expressing *M. bovis* BCG (red channel) and one day of treatment with $MPEO_{113}$-*b*-PCL_{33}-DACCA NPs (**P4**, blue channel). Note the increased NPs-related signal within the location of mycobacteria persistence (white arrows). Scale bar: 10 μm.

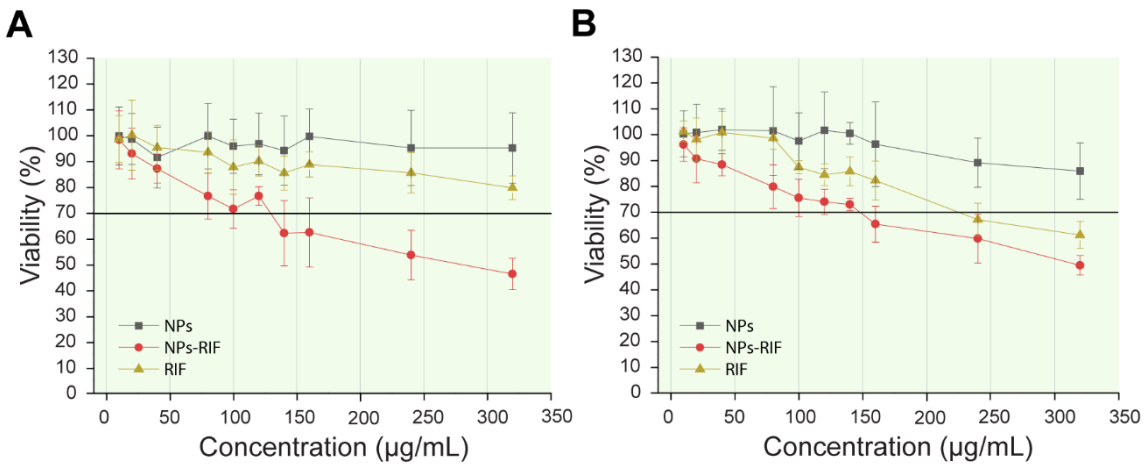

Figure S11. ZF4 cells viability detected by MTT assay. Cells were incubated with different concentrations of RIF-free $MPEO_{113}$-*b*-PCL_{33} (**P4**) NPs, RIF-loaded **P4** NPs (NPs-RIF) and free RIF for 24 h (**A**) and 48 h (**B**). The horizontal lines in the panels indicate a cell viability level where data above is considered non-cytotoxic and below cytotoxic.